

# Streptavidin and Fluorescent Conjugates of Streptavidin

## Quick Facts

### Storage upon receipt:

#### Unlabeled Streptavidin

- $\leq -20^{\circ}\text{C}$
- Desiccate

#### Fluorescent-Dye Conjugates

- $\leq -20^{\circ}\text{C}$
- Desiccate
- Protect from light

#### R-Phycoerythrin, B-Phycoerythrin, Allophycocyanin and Tandem Conjugates

- $2-6^{\circ}\text{C}$
- Do not freeze
- Protect from light

## Introduction

Molecular Probes carries an extensive line of unlabeled and labeled streptavidin products (Table 1), including conjugates of R-phycoerythrin (R-PE), B-phycoerythrin (B-PE) and allophycocyanin (APC) (Table 2). The high affinity of avidin, an egg-white protein, for biotin was first exploited in histochemical applications in the mid-1970s.<sup>1,2</sup> Avidin and its bacterial counterpart, strept-avidin (from *Streptomyces avidinii*), have since become standard reagents for diverse detection schemes.<sup>3</sup> Streptavidin, a nonglycosylated 52,800-dalton protein, reportedly exhibits less nonspecific binding than avidin. However, streptavidin contains a tripeptide sequence Arg-Tyr-Asp (RYD) that apparently mimics the Arg-Gly-Asp (RGD) binding sequence of fibronectin, a component of the extracellular matrix that specifically promotes cellular adhesion.<sup>4</sup> This universal recognition sequence binds integrins and related cell-surface molecules.<sup>5,6</sup> Background problems sometimes associated with streptavidin may be attributable to this tripeptide.

In addition to the products listed in this product information sheet, Molecular Probes prepares conjugates of avidin and NeutrAvidin™ biotin-binding protein, fluorescent conjugates of many species-specific anti-IgG antibodies and conjugates of protein A and protein G. Visit our Web site ([www.probes.com](http://www.probes.com)) for additional information.

**Table 1.** Unlabeled streptavidin and fluorescent-dye conjugates of streptavidin.

Label	Abs *	Em *	Catalog Number
Unlabeled streptavidin	NA	NA	S888
<b>Fluorescent Dye Conjugates</b>			
Alexa Fluor® 350	346	442	S11249
Marina Blue™	365	460	S11221
Cascade Yellow™	402	545	S11228
Alexa Fluor® 405	402	421	S32351
Pacific Blue™	410	455	S11222
Alexa Fluor® 430	434	539	S11237
Fluorescein	494	518	S8
Alexa Fluor® 488	495	519	S11223, S32354
Oregon Green® 488	496	524	S6368
DyeMer™ 488/605	502	609	S32385
DyeMer™ 488/615	502	615	S32386
DyeMer™ 488/630	502	630	S32387
Alexa Fluor® 500	503	525	S32352
Oregon Green® 514	511	530	S6369
Alexa Fluor® 514	518	540	S32353
Alexa Fluor® 532	530	554	S11224
Alexa Fluor® 555	555	565	S21381, S32355
Tetramethylrhodamine	555	580	S870
Alexa Fluor® 546	556	573	S11225
Rhodamine B	570	590	S871
Rhodamine Red™-X	570	590	S6366
Alexa Fluor® 568	578	603	S11226
Alexa Fluor® 594	590	617	S11227, S32356
Texas Red®	595	615	S872
Texas Red®-X	595	615	S6370
Alexa Fluor® 610	612	628	S32359
Alexa Fluor® 633	632	647	S21375
Alexa Fluor® 635	633	647	S32364
Alexa Fluor® 647	650	668	S21374, S32357
Alexa Fluor® 660	663	690	S21377
Alexa Fluor® 680	679	702	S21378, S32358
Alexa Fluor® 700	702	723	S21383
Alexa Fluor® 750	749	775	S21384

\* Approximate absorption (Abs) and fluorescence emission (Em) maxima for conjugates, in nm. Complete spectra for most of these dyes are available at our Web site. † CMNB-caged fluorescein. NA = Not applicable.

### Unlabeled Streptavidin

Streptavidin has four binding sites, permitting a number of techniques in which unlabeled streptavidin can be used to bridge two biotinylated reagents. This bridging method, which is commonly used to link a biotinylated probe to a biotinylated enzyme in enzyme-linked immunohistochemical applications, often eliminates the background problems that can occur when using direct streptavidin-enzyme conjugates.

### Fluorescent Streptavidin Conjugates

Some of the simplest fluorescent detection schemes involving streptavidin entail applying a biotinylated probe to the desired sample and then detecting the bound probe with a fluorescently labeled streptavidin. Fluorescent conjugates of streptavidin are commonly used to localize antigens in cells and tissues<sup>7,8</sup> and to detect biomolecules in immunoassays and DNA hybridization techniques.<sup>9-12</sup>

### R-PE, B-PE, APC and Tandem Conjugates of Streptavidin

Molecular Probes offers R-PE, B-PE and APC conjugates of streptavidin, as well as tandem conjugates of R-PE and APC labeled with long-wavelength Alexa Fluor dyes.

Our tandem conjugates comprise a donor phycobiliprotein, either R-PE or APC, conjugated to longer-wavelength light-emitting fluorescence acceptors. By the process of fluorescence resonance energy transfer (FRET), an energy transfer cascade is established wherein most of the light absorbed by the donor R-PE or APC results in fluorescence of the acceptor dye. This process can be quite efficient, resulting in almost total transfer of energy to the acceptor dye.

Our premium grade R-PE and APC conjugates of streptavidin have been highly purified to ensure that the products are: 1) predominantly 1:1 conjugates of R-PE or APC and streptavidin; 2) free of all unconjugated streptavidin; and 3) mostly free of unconjugated R-PE or APC. The premium grade R-PE and APC conjugates are a fractionation of our S-866 and S-868 products, respectively, intended especially for applications that benefit from additional purity. The purity of each lot is confirmed by analytical HPLC.

Molecular Probes' APC conjugates are prepared from chemically crosslinked APC to avoid dissociation of the molecule into subunits when highly diluted.<sup>13</sup>

## Materials

### Unlabeled Streptavidin

Unlabeled streptavidin (S888) is supplied as a lyophilized powder in a unit size of 5 mg. The specific activity is ~14 U/mg, where one unit is defined as the amount of protein required to bind 1 µg of biotin. Streptavidin is soluble to at least 10 mg/mL dissolved in phosphate-buffered saline (PBS) or other suitable buffer. This lyophilized product is stable for at least three years when stored desiccated at ≤-20°C. Reconstituted solutions are stable for approximately three months with the addition of sodium azide to a final concentration of 2 mM or thimerosal to 0.2 mM. For longer storage, divide solutions into aliquots and freeze at ≤-20°C. AVOID REPEATED FREEZING AND THAWING OF SOLUTIONS.

Table 2. R-PE, B-PE, APC and tandem conjugates of streptavidin.

Label	Abs *	Em *	Catalog Number
<b>R-PE, B-PE and Tandem-R-PE Conjugates</b>			
B-Phycoerythrin (B-PE)	546, 565 †	575	S32350
R-Phycoerythrin (R-PE)	496, 546, 565 †	578	S866, S21388 ‡
Alexa Fluor® 610-R-PE	496, 546, 565 †	630	S20982
Alexa Fluor® 647-R-PE	496, 546, 565 †	668	S20992
Alexa Fluor® 680-R-PE	496, 546, 565 †	702	S20985
Alexa Fluor® 750-R-PE	496, 546, 565 †	771	S32363
<b>APC and Tandem-APC Conjugates</b>			
Allophycocyanin (APC)	650	660	S868, S32362 ‡
Alexa Fluor® 680-APC	650	702	S21002
Alexa Fluor® 700-APC	650	723	S21005
Alexa Fluor® 750-APC	650	775	S21008

\* Approximate absorption (Abs) and fluorescence emission (Em) maxima for conjugates, in nm. † Multiple absorption peaks. ‡ Premium grade.

### Fluorescent Streptavidin Conjugates

Most of the fluorophore-labeled streptavidin products are supplied as lyophilized powder in 1 mg unit sizes. Conjugates with DyeMer™ fluorophores are supplied in 500 µg unit sizes. Certain conjugates are available as 2 mg/mL solutions in PBS, pH 7.2, containing 5 mM sodium azide, in 0.5 mL unit sizes. Peak absorption and emission wavelengths for these products are given in Table 1. The approximate degree of labeling of each conjugate is listed on the product's label. For the lyophilized products, solutions can be made by dissolving the powder in 0.5-1.0 mL of PBS or other suitable buffer. In the lyophilized powder form, the conjugates are stable for at least two years when stored at ≤-20°C. Reconstituted solutions are stable for approximately six months with the addition of sodium azide to a final concentration of 2 mM or thimerosal to 0.2 mM. Fluorescent streptavidin conjugates supplied in solution are also stable for approximately six months. For longer storage, divide solutions into aliquots and freeze at ≤-20°C. PROTECT FROM LIGHT. AVOID REPEATED FREEZING AND THAWING OF SOLUTIONS.

### R-PE, B-PE, APC and Tandem Conjugates of Streptavidin

These products are stable for at least six months when stored undiluted at 2-6°C. DO NOT FREEZE R-PE, B-PE, APC OR TANDEM CONJUGATES. PROTECT FROM LIGHT.

### Streptavidin-R-PE and Streptavidin-B-PE

The R-PE conjugates of streptavidin (S866, S21388) and the B-PE conjugate of streptavidin (S32350) are each shipped as a 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide, in a 1 mL unit size.

### Streptavidin-APC

The allophycocyanin conjugates of streptavidin are each shipped as a 1 mg/mL solution in 0.1 M sodium phosphate, 0.1 M NaCl, pH 7.5, containing 2 mM sodium azide, in a 0.5 mL (S868) or 250 µL (S32362) unit size.

### Tandem Conjugates of Streptavidin

The Alexa Fluor dye-R-PE and Alexa Fluor dye-APC tandem conjugates (Table 2) are shipped as 1 mg/mL solutions in 0.1 M sodium phosphate, 0.1 M NaCl, 2 mM EDTA, pH 7.5, containing 1% glycerol and 5 mM sodium azide, in unit sizes of 100 µL.

### Applications

Streptavidin conjugates are used as secondary detection reagents in histochemical applications, flow cytometry,<sup>14,15</sup> blot analysis and immunoassays. These reagents can also be employed to localize biocytin, biocytin-X, biotin ethylenediamine and Alexa Fluor®, Cascade Blue® or lucifer yellow biocytins — derivatives of biotin that are used as neuroanatomical tracers.<sup>16,17</sup> The following are commonly used methods for employing streptavidin as a secondary detection reagent.

- **Direct Procedure:** A biotinylated primary probe such as an antibody, single-stranded nucleic acid probe or lectin is bound to tissues, cells or other surfaces. Excess protein is removed by washing, and detection is mediated by reagents such as our fluorescent streptavidins or our enzyme-conjugated streptavidins plus substrate.

- **Indirect Procedures:** A biotinylated antibody or oligonucleotide is used to probe a tissue, cell or other surface. This preparation is then treated with unlabeled streptavidin. Excess reagents are removed by washing, and detection is mediated by a biotinylated detection reagent such as fluorescein biotin (B1370), biotinylated R-PE (P811), biotinylated FluoSpheres® microspheres or a biotinylated enzyme (P917) plus a substrate. Alternatively, an unlabeled primary antibody is bound to a cell followed by a biotinylated species-specific secondary antibody. After washing, the complex is detected by the direct or indirect procedures described above.

It is a good practice to centrifuge protein conjugate solutions briefly in a microcentrifuge before use; only the supernatant should then be used for the experiment. This step will eliminate any protein aggregates that may have formed in solution, thereby reducing nonspecific background staining. Because staining protocols vary with the application, appropriate dilutions of conjugates should be determined empirically. For fluorescent dye conjugates of streptavidin, including R-PE, B-PE, APC and tandem conjugates of streptavidin, a final concentration of 1–10 µg/mL is usually satisfactory for most histochemical applications.

### References

1. Proc Natl Acad Sci USA 71, 3537 (1974); 2. Biochim Biophys Acta 264, 165 (1972); 3. "Avidin-Biotin Technology," M. Wilchek and E. Bayer, Eds., Meth Enzymol 184 (complete volume); 4. Biochem Biophys Res Comm 170, 1236 (1990); 5. Eur J Cell Biol 60, 1 (1993); 6. Eur J Cell Biol 58, 271 (1992); 7. J Cell Biol 111, 1183 (1990); 8. Physiol Plantarum 79, 231 (1990); 9. Cytometry 11, 126 (1990); 10. Proc Natl Acad Sci USA 87, 6223 (1990); 11. Science 249, 928 (1990); 12. Anal Biochem 171, 1 (1988); 13. Cytometry 8, 91 (1987); 14. J Microbial Methods 12, 1 (1990); 15. Biochemistry 16, 5150 (1977); 16. J Neurosci 10, 3421 (1990); 17. Brain Res 497, 361 (1989).

### Product List

Current prices may be obtained from our Web site or from our Customer Service Department.

Cat #	Product Name	Unit Size
S888	streptavidin .....	5 mg
S11249	streptavidin, Alexa Fluor® 350 conjugate .....	1 mg
S32351	streptavidin, Alexa Fluor® 405 conjugate .....	1 mg
S11237	streptavidin, Alexa Fluor® 430 conjugate .....	1 mg
S11223	streptavidin, Alexa Fluor® 488 conjugate .....	1 mg
S32354	streptavidin, Alexa Fluor® 488 conjugate *2 mg/mL* .....	0.5 mL
S32352	streptavidin, Alexa Fluor® 500 conjugate .....	1 mg
S32353	streptavidin, Alexa Fluor® 514 conjugate .....	1 mg
S11224	streptavidin, Alexa Fluor® 532 conjugate .....	1 mg
S11225	streptavidin, Alexa Fluor® 546 conjugate .....	1 mg
S21381	streptavidin, Alexa Fluor® 555 conjugate .....	1 mg
S32355	streptavidin, Alexa Fluor® 555 conjugate *2 mg/mL* .....	0.5 mL
S11226	streptavidin, Alexa Fluor® 568 conjugate .....	1 mg
S11227	streptavidin, Alexa Fluor® 594 conjugate .....	1 mg
S32356	streptavidin, Alexa Fluor® 594 conjugate *2 mg/mL* .....	0.5 mL
S32359	streptavidin, Alexa Fluor® 610 conjugate .....	1 mg
S20982	streptavidin, Alexa Fluor® 610-R-phycoerythrin conjugate (Alexa Fluor® 610-R-phycoerythrin streptavidin) *1 mg/mL* .....	100 µL
S21375	streptavidin, Alexa Fluor® 633 conjugate .....	1 mg
S32364	streptavidin, Alexa Fluor® 635 conjugate .....	1 mg
S21374	streptavidin, Alexa Fluor® 647 conjugate .....	1 mg
S32357	streptavidin, Alexa Fluor® 647 conjugate *2 mg/mL* .....	0.5 mL
S20992	streptavidin, Alexa Fluor® 647-R-phycoerythrin conjugate (Alexa Fluor® 647-R-phycoerythrin streptavidin) *1 mg/mL* .....	100 µL
S21377	streptavidin, Alexa Fluor® 660 conjugate .....	1 mg
S21378	streptavidin, Alexa Fluor® 680 conjugate .....	1 mg
S32358	streptavidin, Alexa Fluor® 680 conjugate *2 mg/mL* .....	0.5 mL
S21002	streptavidin, Alexa Fluor® 680-allophycocyanin conjugate (Alexa Fluor® 680-allophycocyanin streptavidin) *1 mg/mL* .....	100 µL
S20985	streptavidin, Alexa Fluor® 680-R-phycoerythrin conjugate (Alexa Fluor® 680-R-phycoerythrin streptavidin) *1 mg/mL* .....	100 µL